readily discerned from the following detailed description of exemplary embodiments thereof especially when read in conjunction with the drawings attached hereto.

Brief Description of the Drawings

In the drawings:

Figure 1 shows the chemical structures of dolastatin 10 (**1a**) and derivatives (**1b-1e**).

Figure 2 shows the *Cryptococcus neoformans* killing kinetics of dolastatin 10 and selected modifications where "killing" is shown as squares (no drug), triangles (1x MIC), inverted triangles (4x MIC), and diamonds (8x MIC) for each compound.

Detailed Description of the Invention

Materials and Methods.

Antifungal agents.

Dolastatin 10 **1a** and modification **1d** (Figure 1) were synthesized as described elsewhere (Pettit et al., 1989, *supra*; Pettit et al., 1996, *supra*; Pettit et al., Antineoplastic agents 365. Dolastatin 10 SAR probes, *Anti-Cancer Drug Design* 1997: In press). Synthesis of modification **1e** is described in U.S. patent 5,663,149 (issued September 2, 1997), and synthesis of modifications **1b** and **1c** are described in pending US applications SN 60/070,879 and 60/091,705. The compounds were reconstituted in sterile dimethylsulfoxide (DMSO) immediately prior to all assays. DMSO alone had no detectable inhibitory effect on any of the tested microbes.

Fungal strains.

Clinical isolates of *C. neoformans* were obtained from patient cerebrospinal fluid, blood, bone marrow, sputum, bronchial lavage and wound infections at the University of Virginia Medical Center. Strains clinically resistant to fluconazole (See: Jessup, C.J. et al, 1997; Poster #F-88, 37th ICAAC, Toronto, Canada) were provided by the Center for Medical Mycology, Case Western Reserve University. Yeast strains (except for *C. albidus* and *C. laurentii*) were maintained by single colony transfer on Sabouraud Dextrose Agar

Broth macrodilution assays were performed with RPMI prepared at pH 5, pH 6 and pH 7, and in RPMI, with and without 50% normal human serum (Lampire Biological Labs). *Cryptococcus neoformans* #90112 was used in each case.

Killing kinetics.

Overnight cultures of *C. neoformans* (#90112) in Ph 7.0 MOPS-buffered RPMI 1640 medium were inoculated into the same medium containing multiples of the broth macrodilution MIC of the antifungal peptides, or an equivalent volume of DMSO. Cultures were shaken at 35°C, and aliquots aseptically removed at various times for dilution plating.

Results and Discussion.

The initial screen for antimicrobial activity, the disk diffusion assay, suggested that dolastatin 10 and four analogs had narrow-spectrum antifungal activity (Table 1). Furthermore, at 100 μg/disk there was no inhibition of the tested bacterial strains (*see*, Materials and Methods, *supra*). The specificity for *C. neoformans* was confirmed by broth macrodilution (Table 2). As with the disk diffusion technique, the parent compound was not growth inhibitory to the related species *C. albidus* and *C. laurentii*. *C. uniguttulatus* and *C. ater*. The MFCs for *C. neoformans* were typically identical or twofold greater than MICs. Exceptions occurred with *C. neoformans* #14116, where MFCs with compounds **1b** and **1c** were sixteenfold greater than MICs. Dolastatin 10 was also fungicidal for strains of *C. neoformans* that were clinically resistant to fluconazole (Jessup et al., supra) (Table 3). As the methyl ester **1d** was the most potent antifungal peptide in broth macrodilution tests, it was tested against 19 clinical isolates (did not include fluconazole-resistant strains) of *C. neoformans*. No resistant clinical isolates were found.

Table 1. Antifungal activity of dolastatin 10 (1a) and modifications (1b-1e) in the disk diffusion assay.

Organism	ATCC#	1a MIC µg/disk	1b MIC µg/disk	1c MIC µg/disk
Cryptococcus neoformans	90112	25-50	3.12-6.25	1.56-3.12
Cryptococcus albidus	66030	>100	>100	
Cryptococcus laurentii	66036	>100	>100	
Candida albicans	90028	>100	>100	>100
Candida glabrata	90030	>100	>100	>100

Organism	ATCC#	1d MIC µg/disk	1e MIC µg/disk
Cryptococcus neoformans	90112	3.12-6.25	25-50
Cryptococcus albidus	66030	>100	
Cryptococcus laurentii	66036	>100	
Candida albicans	90028	>100	>100
Candida glabrata	90030		

Table 2. Antifungal activity of dolastatin 10 (1a) and modifications (1b-1e) in the broth macrodilution assay.

Organism	ATCC#	1a MIC µg/ml	1a MFC µg/ml	1b MIC µg/ml	1b MFC µg/ml	1c MIC µg/ml	1c MFC µg/ml
Cryptococcus neoformans	66031	0.78	1.56	0.78	0.78	0.78	0.78
Cryptococcus neoformans	14116	3.12	6.25	1.56	25	0.78	12.5
Cryptococcus neoformans	32045	0.78	1.56	0.78	0.78	0.78	1.56
Cryptococcus neoformans	90112	0.78	1.56	1.56	3.12	0.78	0.78
Cryptococcus albidus	66030	>50					
Cryptococcus albidus[a]	34140	>50					
Cryptococcus albidus	10666	>50					
Cryptococcus laurentii	66036	>50					
Cryptococcus laurentii	18803	>50					
Cryptococcus laurentii[a]	34142	>50					
Cryptococcus uniguttulatus[a]	34143	>50					
Cryptococcus uniguttulatus	66033	>50					

was no evidence of recovery.

The fungicidal action of four of the peptides was confirmed in killing kinetics experiments (Figure 2) (a paucity of modification **1c** prohibited killing kinetics). In general, killing was concentration dependent between 1x and 4x the MIC, but not between 4x and 8x the MIC. The most dramatic reductions in CFUs were obtained with modification **1d**.

Dolastatin 10 and three of the modifications were available in sufficient quantity to investigate the effects of two host factors, pH and serum, on broth macrodilution MICs and MFCs. The MICs and MFCs increased in acidified RPMI (Table 4). The anticryptococcal activity of modification **1d** was the least affected by lowered pH. Attempts were made to obtain MICs at pH 8, but the strain did not grow in alkaline RPMI.

Table 3. Inhibition of fluconazole-resistant[a] *Cryptococcus neoformans* by dolastatin 10 (1a)

Strain	MIC (μg/ml)	MFC (μg/ml)
94-2406	0.0487	0.0975
95-2792	0.78	3.12
96-2011	0.78	1.56
94-2483	0.195	0.39

[a] Jessup, supra